Simplifying Progress

Optimized Process Performance and Productivity With a Newly Developed Protein A Membrane

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1. Abstract

Intensified processing is achieved using high throughput tools that are most often also single-use, scaled-down and cost-effective. Applying this concept to a mAb capture step will require new high throughput and high productivity materials. We developed a new "convecdiff" base membrane that delivers high mass transfer and high permeability, thus resolving the limitations of diffusional and purely convective materials. MAb processes based on Rapid Cycling Chromatography are enabled, resulting in exceptionally high productivity and reduction of COGs in low batch number processes.

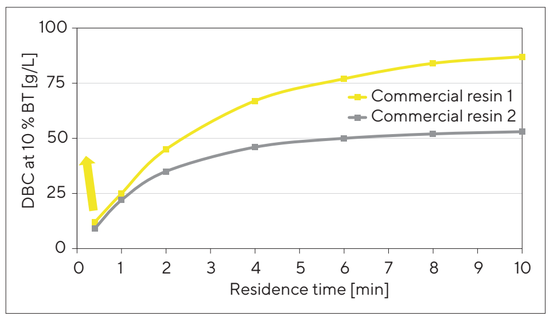
2. Rapid Cycling Chromatogaphy

Rapid Cycling Chromatography (RCC) enables processing of a batch with a high number of small and short cycles compared to resins. The number of cycles can vary and depend on the design of your process. In comparison to resins where one single cycle takes atleast 2 hours, each cycle in RCC takes only 5 — 8 minutes depending on the protocol. This also means that each phase or step within this cycle takes in average only 1 min.

Table 1: Comparison of RCC and traditional chromatography process design

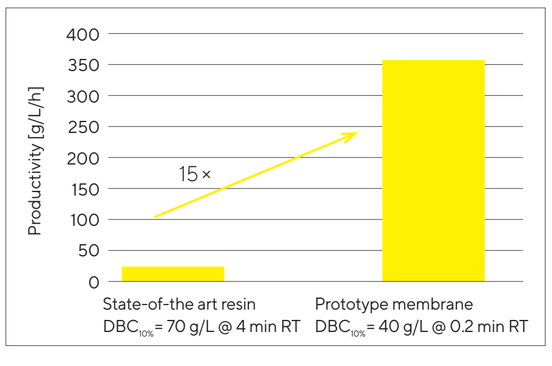
	Rapid Cycling Chromatography	Traditional Chromatography
Cycle n°	~ 30 — 150	4 — 6
Cycle time	~ 5 — 8 mins	> 2 hours
Time of each phase in a cycle	~ 1 min	Several min to hours

3. High $DBC_{10\%}$ at Short Residence Time



We target a high binding capacity at short residence time, which will enable RCC. If we compare this performance to state of the art resins, which are commercially available we see a huge increase in the binding capacity at short residence times. Resins are diffusion limited materials and show a good binding capacity at residence time of 4 to 6 min. Our newly developed membrane overcomes the limitations of diffusional and purely convective materials, therefore we call it "convecdiff".

4. Significant Increase in Productivity



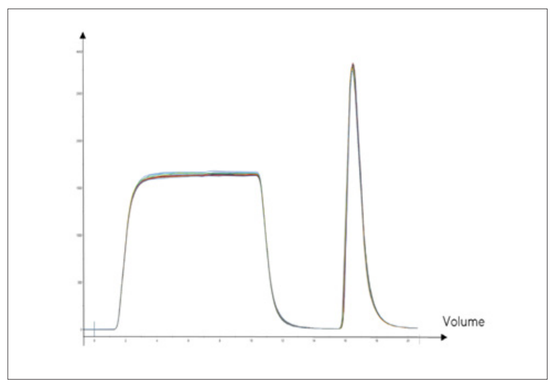
Model input
- 5 CV/MV for washing, elution and equilibration
- No CIP

Rapid Cycling Chromatography based on material with high mass transfer, DBC of 40 g/L (assumed for calculation) and short residence times increase productivity by factor 15 compared to state-of-the-art resin.

5. Lifetime Utilization within One Batch

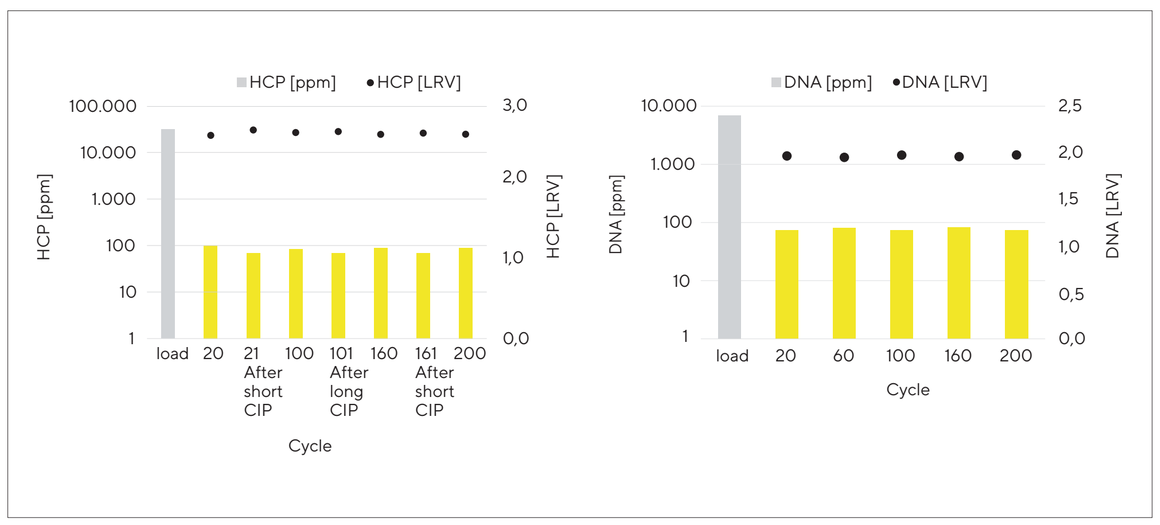
Table 2: Method for bench top system

	Volume [mL]	Flow [mL/min]	Flow [MV/min]		Time [min]
Equilibration	6	5	8		1.2
Load	9	3	5		3
Wash	5	5	8		1
Elution	6	3	5		2
Wash	5	5	8		1
Short CIP	5	3	5	after 20 cycles	1.7
Long CIP	15	1	2	after 100 cycles	15
Wash	20	5	8		4



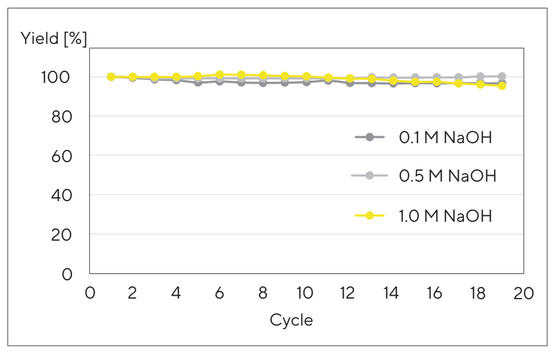
Rapid Cycling Chromatography allows processing of a single-batch with a high number of cycles (lifetime utilization) while a single cycle lasts only 5 — 8 min. Shown in the figure is the overlay of the A280 absorbance of 200 cycles which were performed with the protocol in table 2. A short CIP step after every 20 cycles and a long CIP after 100 cycles was included with only 0.5 M NaOH. This resulted in constant chromatograms over the 200 cycles without any shift in the elution peak. The maximum pressure over the whole cycle study was below 0.2 bar @ 8 MV/min. The large pores of the membrane reduce fouling of the material, which makes this membrane highly suitable for every feed. In addition, the hydrophilic material reduces unspecific binding and lead to very high contaminant removal.

6. Efficient Contaminant Removal



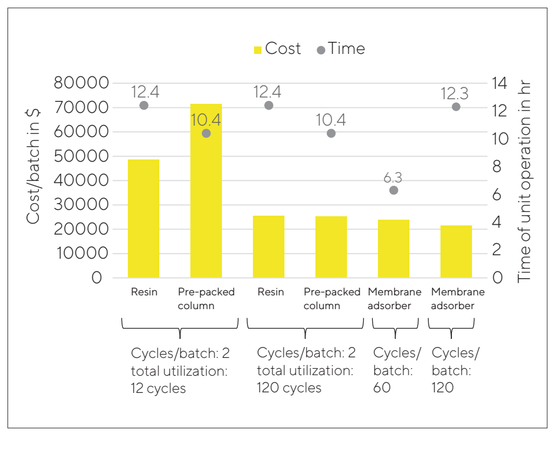
Eluates of the cycles before and after the short or long CIP step (from 5.) were analyzed for HCP and DNA removal and compared to the load. The protein A prototype membrane provides constant HCP and DNA removal over 200 cycles without any big cycle-to-cycle variations. Purification of Cellca 2 mAb feed results in a log reduction of HCPs in the range of > 2.5 and for DNA of > 2.

7. Highest Cleaning and Sanitization Standards



Repetitive purification cycles were performed using 0.1 M, 0.5 M and 1.0 M NaOH as cleaning agent for 15 min/cycle. The protein A prototype membrane showed constant yield of mAb even after 300 min exposure to 1 M NaOH. Thus, meeting highest cleaning and sanitization standards used in bioprocessing.

8. Reduced COGs in Low Batch Number Processes



Model input
- 200 L fed-batch
- Titer: 5 g/L
- Cost: resin: 15 k$/L; equivalent cost asssumed for prepacked column & membrane adsorber: 20 k$/L
- DBC = 40 g/L

A very important benefit of RCC is the reduction of costs during clinical phases. Here, underutilization of protein A resins is the most significant cost driver. Only at higher cycle numbers, do resin based processes become economical. When using membranes, early investments are reduced through lifetime utilization during a single batch. In addition, process time can be reduced by adapting cycle number according to process layout.

9. Summary

Highly flexible processes using Rapid Cycling Chromatography for mAb capture are enabled by cycle times of 5 to 8 minutes and residence times of seconds. Our new protein A membrane exhibits excellent alkaline stability, allowing use of 1 M NaOH for effective bioburden and process risk control. The membrane can be used for more than 200 cycles per batch at low pressure drops, has low fouling propensity, and delivers state-of-the-art performance in terms of contaminant removal and recovery. Through full lifetime utilization within low batch number processes (e.g. for trial material) and via miniaturization of full scale manufacturing processes, this new "convecdiff" membrane offers new possibilities for production of clinical and commercial drug substance.

Table 3: Benefits of Rapid Cycling Chromatography using membrane adsorber

Clinical Phase	Commercial Scale
Lifetime utilization per batch	Low bioburden
Cost-effective	Small footprint (miniaturization)
No storage	Fast processing
Low CAPEX	Simplification
Plug & play	Flexibility
	No storage
	No cleaning validation